GenepHlow™ DNA Cleanup Maxi Kit Quick Protocol

For research use only

Catalogue Number

DFM002, DFM010, DFM025

Instruction Manual Download

When using this product for the first time, or if you are unfammiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

Gel Extraction Protocol

1. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 5 g of the gel slice** to a 15 ml centrifuge tube. Add **8 ml of Gel/PCR Buffer** to the sample then mix by vortex. Incubate at 55-60ºC for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of the mixture has turned from yellow to purple, add **200 µl of 3M Sodium Acetate (pH5.0)** and mix thoroughly. This will adjust pH and the color will return to yellow. Cool the dissolved sample mixture to room temperature.

2. DNA Binding

Place a **DM Column** in a 50 ml centrifuge tube. Transfer **6 ml of the sample mixture** to the **DM Column** (DO NOT load more than 6 ml of the sample mixture to the DM Column to prevent spilling during centrifugation). Centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube. Transfer the remaining sample mixture to the same **DM Column** and centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube.

3. Wash

Add **6 ml of W1 Buffer**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer** (make sure absolute ethanol was added) into the CENTER of the **DM Column** and let stand for 3 minutes at room temperature. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer** (make sure absolute ethanol was added) into the CENTER of the **DM Column**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Centrifuge at 5-6,000 x g for 5 minutes to dry the column matrix.

4. DNA Elution

Transfer the dried **DM Column** to a new 50 ml centrifuge tube. Add **0.5-1 ml of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 5-6,000 x g for 5 minutes at room temperature to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DM Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the DM Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the DM Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Enzymatic Reaction Protocol

1. Sample Preparation

Transfer up to **2 ml of reaction product** to a 15 ml centrifuge tube. Add **5 volumes of Gel/PCR Buffer** to the sample then vortex. If the mixture has turned from yellow to purple, add **200 µl of 3M sodium acetate (pH5.0)** and mix thoroughly. This will adjust pH and the color will return to yellow.

2. DNA Binding

Place a **DM Column** in a 50 ml centrifuge tube. Transfer **6 ml of the sample mixture** to the **DM Column** (DO NOT load more than 6 ml of the sample mixture to the DM Column to prevent spilling during centrifugation). Centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube. Transfer the remaining sample mixture to the same **DM Column** and centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube.

3. Wash

Add **6 ml of Wash Buffer** (make sure absolute ethanol was added) into the CENTER of the **DM Column** and let stand for 3 minutes at room temperature. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer** (make sure absolute ethanol was added) into the CENTER of the **DM Column**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Centrifuge at 5-6,000 x g for 5 minutes to dry the column matrix.

4. DNA Elution

Transfer the dried **DM Column** to a new 50 ml centrifuge tube. Add **0.5-1 ml of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 5-6,000 x g for 5 minutes at room temperature to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DM Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the DM Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the DM Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Kit Components

Component	DFM002	DFM010	DFM025
Gel/PCR Buffer	25 ml	125 ml	275 ml
3M Sodium Acetate (pH5.0)[1]	N/A	2 ml	2 ml
W1 Buffer	15 ml	75 ml	175 ml
Wash Buffer[2] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	2 ml	12 ml	30 ml
DM Columns	2	10	25

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely or following enzymatic product reaction, the pH is too high. 3M Sodium Acetate (pH5.0) can then be added to adjust pH and the color will return to yellow.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Storage

Dry at room temperature (15-25ºC)